PROJECT NUMBER: 1730
PROJECT TITLE: Plant, Cell & Tissue Culture Research
PROJECT LEADER: I. L. Uydess
PERIOD COVERED: April, 1988

I. TOBACCO IDENTICAL PRESERVATIVES

A. Objective: To develop procedures and to establish microbiological screens for the evaluation of new, nature-identical preservatives as replacements for and/or as adjuncts to propylparaben.

B. Status and Results:

1. Phase I, Preservative Screens:

a. Agar-Inclusion Assay:

Seven fatty acids ranging in chain length from C-6 to C-14 were tested in the scaled-down agar inclusion assay. The antimicrobial activity of these compounds was found to increase with increasing chain length up to and including Decanoic (C-10) and Undecylenic (C-11) acids, both of which completely inhibited microbial growth at 100 µg/ml. This is considerably better than the results achieved with propylparaben which inhibits bacterial growth at 300 - 500 µg/ml in this same assay system. The C-14 compound that was tested, Myristic (Tetradecanoic) Acid, did not inhibit bacterial growth completely until 5000 µg/ml.

b. Shake Flask Assay:

Two naturally-occurring compounds, a C-9 fatty acid (Nonanoic Acid) and a tobacco-identical sucrose ester extracted from Oriental tobacco were evaluated for antimicrobial activity in the Phase I (Nutrient Broth) shake flask assay. One hundred and fifty µg/ml of propylparaben in PG was used as the control. One hundred µg/ml of Nonanoic Acid reduced the growth of the target organism (B. coagulans) 70% compared to that of the preservative-free control. The control level of propylparaben (150 µg/ml) commonly used in these same experiments reduced the growth of the target organism 40% - 50%. Two hundred and fifty µg/ml of nonanoic acid totally inhibited the growth of the B. coagulans. The sucrose ester fraction, on the other hand, was observed to be even more effective as an antimicrobial agent since it completely inhibited growth at 100 µg/ml.

2. Microbiological Investigations:

a. Phase II screen: Passage of the mixed culture of bacteria isolated from laboratory-spoiled Park 500 SEL continued. Variations in apparent growth rate, final pH and relative proportion of each of the (4 - 5) different colony types were observed to occur during the spoilage cycle in each

passage. These variations in population dynamics and chemistry of the spoiling SEL are thought to be the result of subtle and as yet, ill-understood metabolic interdependencies which exist among the organisms within the mixed culture. Each of the colony morphologies present in the mixed culture have been reisolated in an attempt to establish a single-isolate, SEL (Phase II) preservative screen, as well as to further investigate each isolate's contribution to the observations that have been made.

b. Spoilage of Starch-Based Adhesives: Nonanoic acid was incorporated into samples of a starch-based adhesive (National Starch and Chemical Corp.) and its antimicrobial activity evaluated. Duplicate aliquots of this material containing 0 to 1000 µg/ml nonanoic acid were dispensed asceptically into sterile petri dishes containing 10 mls of starch-based adhesive and exposed to the air in the Manufacturing Center glue room for 2 hours. The samples were then sealed, returned to the laboratory and incubated overnight at 21°C. Trypticase Soy Agar plates (+/- nonanoic acid) exposed to the air in the glue room and unexposed, adhesive-containing plates (sealed in the laminar flow hood) were included as additional controls. After 24 hours of incubation, a variety of mold and bacteria were observed on the control TSA plates (- nonanoic acid). Mold, but no bacteria were found on the TSA plates containing nonanoic acid at concentrations of 500 µg/ml and above. Mold was also observed on all of the starch-based adhesive plates, even those not exposed to the air in the glue room. Thus, there was a mold contaminant in the starch-based adhesive as supplied by the manufacturer that was not sensitive to the concentrations of nonanoic acid that were used. In a previous experiment (see March monthly), propylparaben was found to be effective against the mold present in those experiments.

C. **Conclusions:** None to be reported at this time.

D. **Plans** (May, 1988):

1. Additional fatty acids with chain lengths between C-10 and C-14 will be tested in the Phase I preservative screens.

2. Further work will be done on the development of a reproducible, single-isolate Phase II (SEL) screen.